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# Copper(II) and zinc(II) complexes of mono- and tri-linked azacrown macrocycles: synthesis, characterization, X-ray structure, phosphodiester hydrolysis and DNA cleavage

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## Abstract

A new tri-linked azacrown macrocycle (**L2**) was synthesized from mono macrocycle analogue (**L1**) by Williamson etherification and characterized by FT-IR, $^{1}$H NMR, $^{13}$C NMR, DEPT $^{13}$C NMR, MS, and elemental analysis. The reaction of copper(II) and zinc(II) salts yielded corresponding complexes and formulated as Cu**L1**$Cl_2$ (**1**), Cu**L1**$(NO_3)_2.3H_2O$ (**2**), $Cu_2$**L2**$(NO_3)_4.4H_2O$ (**3**), Zn**L1**$(OAc)_2$ (**4**) and $Zn_3$**L2**$(OAc)_6.3H_2O$ (**5**). Mono and trinuclear zinc(II) complexes **4** and **5**, respectively, have been tested as catalysts for hydrolysis of 2-hydroxylpropyl-4-nitrophenyl phosphate (HNPP). At pH 8.5 the mononuclear complex **4** was found to be inactive. In contrast, trinuclear complex **5** was hydrolyizing phosphodiester and the reaction was up to 35–fold faster than the unpromoted reaction. Mono and dinuclear copper(II) complexes **2** and **3** cleave plasmid pG2 DNA by using an oxidative mechanism under aerobic conditions. Dinuclear copper(II) complex **3** showed a much higher cleavage efficiency than its mononuclear analogue **2** at the same $Cu^{2+}$ concentration. The X-ray structure of **1** is reported. In this complex, the Cu(II) is bound by three amine nitrogens from the macrocyclic ligand **L1** and two chloride anions as distorted trigonal bipyramidal geometry.

## Keywords

Macrocycle; Phosphate ester hydrolysis; DNA cleavage; Zinc(II); Copper(II)

## Introduction

In recent years, there has been a considerable interest in the design and synthesis of supermolecular receptor that bind both small molecules and ions [1]. One category of this type of compounds involves linked macrocyclic ring systems. A variety of linked macrocycles incorporating crowns [2, 3], azathia crowns [4], azathia macrocycles [5-7], cyclams [8], and oxaaza macrocycles [9], have been synthesized. Linked macrocycles which are able to incorporate two or more metal ions offer the prospect of generating unusual electronic and chemical properties [10-14]. These systems exhibit characteristic properties such as magnetic exchange between adjacent metal ions [15, 16], induction of Z-DNA [17], tendency to undergo multi-electron redox processes [18,19], and formation of sandwich configuration type geometries with particular alkali ions [2]. Binuclear linked cyclam macrocycles have been demonstrated to inhibit HIV strains with low levels of cytotoxcity [20-22]. Also several binuclear copper(II) and zinc(II) complexes have been shown catalysis of phosphate esters hydrolysis and DNA cleavage [23, 24].

Previously, we have reported the synthesis of two pendant-armed azacrown macrocycles [25, 26]. In particular, our focus has been on macrocycle **L1** (Scheme 1) that contains the hydroxyl group in the macrocyclic C-backbone because this group can potentially be attached to other substrates after a selective protection of the secondary amines by *tert*-butyloxycarbonyl (Boc) as a flexible protecting group [27, 28]. In an extension of these studies we now present a comparative investigation of aspects of the complexation behavior of new tri-linked macrocycle **L2** and mono macrocycle analogue **L1** toward Zn(II) and Cu(II) metal ions. Also we studied in this paper the kinetics of HNPP hydrolysis and DNA cleavage, in order to study the influence of the nuclearity of complexes.

## Experimental Section

**General procedures.** All chemicals were from Aldrich, Merck and Fluka and used without further purification. 1,3,5-trisbromomethylbenzene was prepared according to published procedure [29]. Reactions were monitored by TLC on Merck silica gel and aluminum plates and some organic reactions were performed under $N_2$. Column Chromatography was carried out with Merck silica gel (> 230 mesh) and Aldrich neutral aluminum oxide (100-300 mesh). NMR spectra were recorded on a Bruker AV2 (400 MHz) and AV1 (500 MHz) spectrometer. Chemical shifts are relative to residual solvent protons or TMS as references. Uv-Vis absorption spectra were recorded by using UV-Vis Cary 500 Version No 8.01 and UV-Vis Cary 50 Version No 1.00 spectrometer. Infrared spectra were measured with a Perkin Elmer FT-IR spectrophotometer. Electrospray ionization spectra (ESI-Mass) were obtained on a Esquire HCT Spectrometer from Bruker (Bremen, Germany) in the Institute of Inorganic Chemistry at the University of Zurich. Elemental analyses were performed on a Leco CHNS-932 elemental analyzer.

**Phosphodiester hydrolysis.** HNPP was freshly prepared according to published procedure [30]. Hydrolysis of HNPP was monitored by the following the visible absorption change at 400 nm ($\varepsilon$ = 18500 $M^{-1}$ $cm^{-1}$) due to the release of p-nitrophenolate anion ($PNP_{ate}$). The activity of the complex was determined by the initial rate method. In a typical kinetic experiment, freshly prepared HNPP stock solution in water with certain concentration was added to the solution of complex. The solution was

buffered with cacodylate and the ionic strength was maintained with 0.1 mM KCl. The final volume was 1 mL. Each solution put at 40 °C during 120 min inside the UV-Vis instrument at 400 nm to obtain kinetic data in different conditions. Pseudo-first-order rate constants for unpromoted and promoted reactions ($k_{uncat}$, $s^{-1}$, $k_{cat}$, $s^{-1}$) were measured by following the increase in absorbance for different HNPP concentrations.

**Cleavage of plasmid DNA.** pG2 DNA (650 ng/µL) in cacodylate buffer (0.1 mM, pH 7.5) containing (50 mM NaCl, pH 7.4) was treated with different concentrations copper(II) complexes **2** and **3** and 1 µL of 20 mM of 3-mercaptopropionic acid (MPA) to yield a total volume of 10 µL. The mixtures were then incubated for 16 h at 37 ˚C. The reaction was quenched by the addition of 2 µL blue dye (50 mM) and then the resulting solutions were loaded on a 1 % agarose gel. Electrophoresis was carried out at 70 mV for 3 h in a TAE buffer (40 mM Tris acetate / 1 mM EDTA pH 8). Bands were visualized under transilluminator light and photographed.

## Syntheses

**Synthesis of BL1.** A solution of $(Boc)_2O$ (6.90 g, 31.6 mmol) in MeOH (30 mL) was added dropwise during 10 min at 0 ˚C to a solution of **L1** (2.35 g, 6.3 mmol) in MeOH (40 mL) under a nitrogen atmosphere. The mixture was stirred at r.t. for 24 h. Water was added to the solution and the stirring continued at 40 ˚C for 2 h. The solvent was removed in vacuum and the residue was purified by chromatography on silica gel. Elution was started with 1:10 EtOAc/n-hexane. The polarity of eluent was gradually increased to 7:10 EtOAc/n-hexane. The product was obtained as a white solid; m.p.: 78-

83 ˚C; yield: 3 g (71 % based on **L1**); $R_f$ = 0.5 (7:10 EtOAc/n-hexane). FT-IR (KBr): 3440 m (OH), 2976 s, 2933 s ($CH_{aliphatic}$), 1708 s (C=O), 1601 m, 1456 s, 1415 s ($C=C_{aromatic}$), 1241 s $(C\text{-}O\text{-}C)_{asym}$, 1160 s $(C\text{-}O\text{-}C)_{sym}$, 1034 m (C-N), 754 s $(=C\text{-}H_{aromatic})_{o.o.p}$ $cm^{-1}$. Elemental analysis: Anal. Calc. for $C_{36}H_{53}O_9N_3$: C, 64.36; H, 7.95; N, 6.25. Found: C, 64.23; H, 7.60; N, 6.23 %. MS (ESI): m/z (%) = 694 (100 $[M+Na]^+$). $^1H$ NMR (400 MHz, $CDCl_3$): δ 1.39 (s, 9H, $C(CH_3)_3$), 1.48 (s, 18H, $C(CH_3)_3$), 2.93 - 3.17 (br m, 8H, $(NCH_2CH_2N)$), 4.12 - 4.38 (br m, 8H, $ArCH_2N$, $ArOCH_2$), 4.66 (br s, 1H, CH), 6.95-7.01 (q, 4H, J= 8 Hz, ArH), 7.27-7.31 (m, 4H, ArH) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 28.4 ($C(\mathbf{C}H_3)_3$), 28.5 ($C(\mathbf{C}H_3)_3$), 45.7 ($NCH_2CH_2N$), 68.5 ($ArOCH_2$), 68.8 ($ArCH_2N$), 79.4 (CHOH), 80.1 ($\mathbf{C}(CH_3)_3$), 112.5 (Ar), 121.7 (Ar), 128.9 (Ar), 130.4 (Ar), 155.1 (Ar), 156.0 (CO), 156.6 (Ar) ppm.

**Synthesis of BL2.** To a stirring suspension of NaH (60 %), (0.53 g, 13.4 mmol) in dry THF (50 mL) under a nitrogen atmosphere, a solution of **BL1** (1.00 g, 15.0 mmol) in dry THF (20 mL) was added at r.t. then 1,3,5–tris (bromomethyl)benzene (0.18 g, 5.0 mmol) was dissolved in dry THF (10 mL) and added dropwise to the above solution during 10 min. The temperature was increased and the reaction was refluxed over night. The solvent was removed under reduced pressure and the solid was partitioned between $CH_2Cl_2$ (200 mL) and brine (200 mL). The organic phase was separated and dried with magnesium sulfate and the solvent was evaporated under reduced pressure with rotary evaporator. The oily compound was purified by chromatography on silica gel. Elution was started with n-pentane. The polarity of eluent was increased gradually to 3:5 EtOAc/n-pentane. The product was obtained as a white solid; yield: 0.4 g (40 % based

on **BL1**); $R_f$ = 0.5 (3:5 EtOAc/n-pentane). FT-IR (KBr): 2976 s, 2932 s ($CH_{aliphatic}$), 1692 m (C=O), 1602 s, 1456 s, 1415 s ($C=C_{aromatic}$), 1243 s $(C\text{-}O\text{-}C)_{asym}$, 1162 s $(C\text{-}O\text{-}C)_{sym}$, 1048 m (C-N), 755 s $(=C\text{-}H_{aromatic})_{o.o.p}$ $cm^{-1}$. Anal. Calc. for $C_{117}H_{165}O_{27}N_9$: C, 65.99; H, 7.81; N, 5.92. Found: C, 65.93; H, 7.60; N, 5.89 %. MS (ESI-positive ion mode): m/z (%) = 2151 (100 $[M + Na]^+$), MS (ESI-negative ion mode): m/z (%) =2173 (100 $[M + HCOO]^-$ ). $^1H$ NMR (400 MHz, $CDCl_3$): δ 1.43, 1.46 (two distinctively singlets, 81H, $C(CH_3)_3$), 3.10 - 3.26 (br m, 24H, $NCH_2CH_2N$), 4.24 - 4.29 (br m, 12H, $ArOCH_2$, 3H, CHO), 4.46 (br s, 12H, $ArCH_2N$), 4.79 (s, 6H, $ArCH_2O$), 6.91 (d, 6H, J= 8Hz ArH), 6.96 (t, 6H, J= 4Hz, ArH), 7.22 - 7.26 (m, 12H, ArH), 7.35 (s, 3H, core ArH) ppm. $^{13}C$ $\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 28.4 ($C(CH_3)_3$), 45.2, 46.2 ($NCH_2CH_2N$), 67.7, 68.6 ($ArCH_2N$, $ArOCH_2$), 71.5 ($ArCH_2O$ core), 76.4(CHO), 79.5, 79.8 ($C(CH_3)_3$), 111.9 (Ar), 121.4 (Ar), 126.3 (Ar), 128.6 (Ar), 129.7 (Ar core), 130.3 (Ar), 138.6 (Ar core), 155.1 (Ar), 155.8 (CO), 156.3 (Ar) ppm.

**Synthesis of L2. BL2** (0.6 g, 0.3 mmol) was added to a stirred mixture of dichloromethane (30 mL) and trifluoroacetic acid (30 mL). The mixture was stirred at r.t for 2 h. After removal of the solvent, methanol was added and then evaporated again to dryness. The mixture was partitioned between aqueous sodium carbonate (15 %, 50 mL) and chloroform (30 mL). The combined organic phases were dried over magnesium sulfate, and the solvent removed to yield **L2** as creamy solid; yield (0.22 g, 64 %); m.p.: 148-152 ˚C; $R_f$ = 0.3 (1:10 conc. $NH_3$-MeOH). FT-IR (KBr): 3350 w (NH, OH), 2875 m ($CH_{aliphatic}$), 1601 m, 1491 s, 1450 s ($C=C_{aromatic}$), 1237 s $(C\text{-}O\text{-}C)_{asym}$, 1109 m $(C\text{-}O\text{-}C)_{sym}$, 1045 m (C-N), 751 s $(=C\text{-}H_{aromatic})_{o.o.p}$ $cm^{-1}$. Anal. Calc. for $C_{72}H_{93}O_9N_9.1.5CHCl_3.1.5H_2O$: C, 61.53; H, 6.85; N, 8.79. Found: C, 61.43; H, 7.00, N,

8.63 %. MS (ESI): m/z (%) = 1228 (100 $[M + H]^+$), 615 (4 $[M + 2H]^{2+}$). $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.46 - 2.55 (m, 24H, $NHCH_2CH_2NH$), 2.79 (br s, 12H, NH, $H_2O$), 3.70 - 3.78 (dd, 12H, J = 12, 12Hz, $ArCH_2N$), 4.16 - 4.26 (m, 15H, $ArOCH_2$, CHO), 4.65 (s, 6H, $ArCH_2$ core), 6.85 - 6.89 (m, 12H, ArH), 7.12 (dd, 6H, J = 0, 0Hz ArH), 7.19 - 7.22 (m, 12H, ArH, ArH core) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 48.8, 48.9 ($NHCH_2CH_2NH$), 50.5 ($ArCH_2N$), 67.5 ($ArOCH_2$), 72.3 ($ArCH_2O$ core), 76.9 (CHO), 111.9 (Ar), 121.2 (Ar), 126.8 (Ar), 127.0 (Ar), 129.1 **(**Ar), 131.1 (Ar core), 138.7 (Ar core), 157.1 (Ar) ppm. $^{13}C\{DEPT, 135\}$ (100 MHz, $CDCl_3$): Aliphatic δ = $CH_2$; 48.8, 48.9, 50.5, 67.5, 72.3, aliphatic CH; 76.9, aromatic CH; 111.9, 121.2, 127.0, 129.1, 131.1 ppm.

**Metal complexes, general procedure**

A methanol solution (10 mL) of ligand (1 mmol) was added slowly to a 10 mL hot methanol solution of metal salt (1 mmol with **L1** and 3 mmol with **L2**) with stirring during 5 min. The solution was stirred and refluxed for 2 h. The solvent was removed by using a rotary evaporator. The resultant solid products were washed by diethylether and acetone and collected by filtration.

**$CuL1Cl_2$ (1).** Blue precipitates. Recrystallized in methanol/1-butanol. Yield (71 % based on **L1**). Anal. Calc. for $C_{21}H_{29}CuCl_2N_3$: C, 49.86; H 5.78; N, 8.31. Found: C, 49.65; H, 5.67; N, 8.27 %. FT-IR (KBr, $cm^{-1}$): 3372 s (OH), 3247 s (NH), 3095 w ($CH_{aromatic}$), 2935 m, 2879 m ($CH_{aliphatic}$) 1602 s, 1587 s, 1492 s ($C=C_{aromatic}$), 1235 s $(C\text{-}O\text{-}C)_{asym}$, 1120 m $(C\text{-}O\text{-}C)_{sym}$, 1053 (C-N), 771 s $(=C\text{-}H_{aromatic})_{o.o.p}$.

**$CuL1(NO_3)_2.3H_2O$ (2).** Blue precipitates, Recrystallized in methanol. Yield (79 % based on **L1**). Anal. Calc. for $C_{21}H_{29}CuN_5O_9.3H_2O$: C, 41.14; H, 5.75; N, 11.42. Found: C, 41.16; H, 5.67; N, 11.27 %. FT-IR (KBr, $cm^{-1}$): 3500 s (OH), 3300 m (N-H), 2900 m ($CH_{aliphatic}$), 1602 s, 1499 s ($C=C_{aromatic}$), 1400 s ($NO_3^-$), 1240 s $(C\text{-}O\text{-}C)_{asym}$, 1020 s $(C\text{-}O\text{-}C)_{sym}$, 750 s $(=C\text{-}H_{aromatic})_{o.o.p}$.

**$Cu_2L2(NO_3)_4.4H_2O$ (3).** Blue precipitates. Recrystallized in methanol/water. Yield (43 % based on **L2**). Anal. Calc. for $C_{72}H_{93}Cu_2N_{13}O_{21}.4H_2O$: C, 51.61; H, 6.07; N, 10.87. Found: C, 51.59; H, 5.91; N, 10.72 %. FT-IR (KBr, $cm^{-1}$): 3436 m (OH), 3225 m (N-H), 2928 m, 2879 m ($CH_{aliphatic}$), 1602 m, 1493 s, 1453 s ($C=C_{aromatic}$), 1384 s ($NO_3^-$), 1237 s $(C\text{-}O\text{-}C)_{asym}$, 1114 m $(C\text{-}O\text{-}C)_{sym}$, 1049 m (C-N), 759 s $(=C\text{-}H_{aromatic})_{o.o.p}$.

**$ZnL1(CH_3COO)_2$ (4).** Yellow precipitates. Recrystallized in methanol/water. Yield (71 % based on **L1**). Anal. Calc. for $C_{25}H_{35}N_3O_7Zn$: C, 54.11; H, 6.36; N, 7.57. Found: C, 53.89; H, 6.48; N, 7.34 %. MS (ESI): m/z (%) = 434 (100 $[Zn + L1 - H]^+$), 371 (66 $[L1 + H]^+$). FT-IR (KBr, $cm^{-1}$): 3450 m (OH), 3247 s (NH), 2930 s, 2876 s ($CH_{aliphatic}$), 1601 s, 1583 s, 1493 s ($C=C_{aromatic}$), 1404 m ($COO_{acetate}$), 1237 s $(C\text{-}O\text{-}C)_{asym}$, 1117 s $(C\text{-}O\text{-}C)_{sym}$, 757 s $(=C\text{-}H_{aromatic})_{o.o.p}$.

**$Zn_3L2(CH_3COO)_6.3H_2O$ (5).** Yellow precipitates. Recrystallized in methanol. Yield (26 % based on **L2**). Anal. Calc. for $C_{84}H_{111}N_9O_{21}Zn_3.3H_2O$: C, 55.04; H, 6.43; N, 6.88. Found: C, 55.05; H, 6.39; N, 6.53 %. FT-IR ($cm^{-1}$): 3432 w (OH), 3250 m (NH), 2932 s ($CH_{aliphatic}$), 1603 s, 1588 s, 1494 s, 1454 s, ($C=C_{aromatic}$), 1404 m ($COO_{acetate}$), 1291 s ($C\text{-}O_{acetate}$), 1244 s $(C\text{-}O\text{-}C)_{asym}$, 1122 s $(C\text{-}O\text{-}C)_{sym}$, 1050 m (C-N), 759 s $(=C\text{-}H_{aromatic})_{o.o.p}$.

**X-ray crystallographic analysis.** Blue crystals of **1** were obtained from methanol/1-butanol (1:1) by slow evaporation. Data were collected at 100 K using the APEX2 suite of programs on a Bruker Smart APEX CCD diffractometer with monochromated Mo $K_\alpha$ radiation (λ = 0.71073 Å) using the ω scan mode [31]. Cell refinement, data reduction and absorption corrections were carried out using APEX2. The structure was solved by direct methods using SHELXTL 6.14 and was refined by full-matrix least-squares calculations on $F^2$ with SHELXTL 6.14 [32, 33]. All H atoms were placed in calculated positions (C–H = 0.99 Á, N–H = 0.92 Á) and were refined using a riding model with an isotropic displacement parameter 1.5 (methyl) or 1.2 times (all others) that of their carrier atoms. Details of the X-ray experiments and crystal data are summarized in Table 1.

**Table 1**

Crystal Data and Structure Refinement Parameters for **1**

| | |
|---|---|
| Formula | $C_{21}H_{29}Cl_2CuN_3O_3$ |
| Formula weight | 505.92 |
| Crystal description | Plate, blue |
| Crystal size (mm) | 0.29 × 0.27 × 0.11 |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| Temperature (K) | 100(2) |
| a (Å) | 10.9242(15) |
| b (Å) | 8.3509(12) |
| c (Å) | 23.681(3) |
| β (°) | 98.869(2) |
| V ($Å^3$) | 2134.5(5) |
| Z | 4 |
| F(000) | 1052 |
| $D_x$($Mg\ m^{-3}$) | 1.574 |
| Radiation type (λ, Å) | Mo $K_\alpha$, 0.71073 |
| μ ($mm^{-1}$) | 1.303 |
| θ range (°) | 1.74- 28.28 |
| Index ranges | -14 ≤ h ≤ 14 |
| | -11 ≤ k ≤ 11 |
| | -31 ≤ l ≤ 31 |
| Max. and min. transmission | 0.8700, 0.7038 |
| Data / restraints / parameters | 5304, 0, 272 |
| Goodness-of-fit on $F^2$ | 1.018 |
| Reflections collected | 21037 |
| Independent reflections | 5304 |
| $R_{int}$ | 0.0369 |
| Final R indices [I > 2σ(I)] | R1 = 0.0321, wR2 = 0.0758 |
| Final R indices (all data) | R1 = 0.0405, wR2 = 0.0810 |
| Largest diff. Peak and hole (e $Å^3$) | 0.547 and -0.415 |

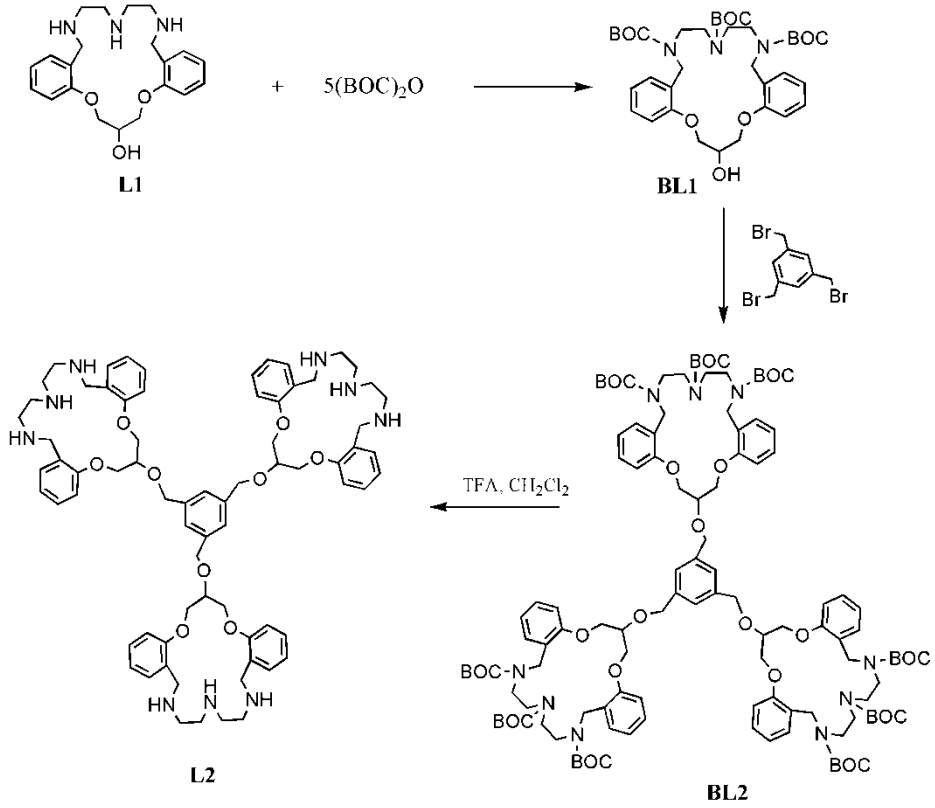
## Results and discussion

The synthesis route for obtaining the target compounds is depicted in Scheme 1. The starting macrocycle **L1** was produced by the reaction of 2-[3-(2-formyl phenoxy)-2-

hydroxyl propoxy]benzaldehyde and diethylenetriamine according to the published procedure [26]. The reaction of **L1** with di-tert-butyl dicarbonate $(Boc)_2O$ in methanol produced the N-protected macrocycle **BL1** in good yield 71 % after column chromatography. After conversion of **L1** into **BL1**, the strong peak for carbonyl group appeared in the FT-IR spectrum at 1708 $cm^{-1}$. Also in the $^1H$ NMR spectrum of **BL1** the aliphatic protons of $CH_3$ in the tert-butyl groups appeared as singlets at 1.39 and 1.48 ppm respectively. The two tert-butyl groups experience different environments. They correspond to the two peaks in the $^{13}C$ NMR at 28.4 and 28.5 ppm.

Synthesis of target compound **L2** started by alkylation of **BL1** as shown in Scheme 1. **BL1** was deprotonated with NaH as a base in dry THF and the resulting deprotonated **BL1** treated with 1,3,5-tribromomethylbenzene afforded the tri-linked N-protected macrocycle **BL2** in 40 % yield. ESI-MS of that showed the expected peaks for the $[M+Na]^+$ and $[M + HCOO]^-$ molecular ions at m/z = 2151 and 2173, respectively. Interpretation of the $^1H$ NMR spectra of the Boc derivatives was complicated by signal broadening due to the slow rotation around the amide bonds. This is no longer the case for the final product **L2** after deprotection **BL2** by using trifluoroacetic acid. The ESI-MS spectrum provided evidence for the structure of **L2** with the expected peak of $[M+H]^+$ at m/z = 1228 (Fig. S1). In the $^1H$ NMR spectrum diagnostic singlet absorptions were observed for the methylene groups of linker arms and aromatic core protons at $\delta$ = 4.65 and 7.22 ppm, respectively (Fig. S2). The $^{13}C$ NMR also confirmed the presence of the triply linked core with one benzylic carbon signal at 72.3 ppm and two aromatic carbon signals at 131.1 and 138.7 ppm (Fig. S3). In addition, $^{13}C$ DEPT NMR for **L2** clearly

showed all aliphatic and aromatic -$CH_2$-, and -CH- groups (Fig. S4). Elemental analysis of **L2** supports the formulation of compound.




**Scheme 1.** Synthetic routes to obtain intermediates **BL1**, **BL2** and **L2** as product.

The reaction of Zinc(II) and Copper(II) salts with **L1** and **L2** yielded corresponding complexes. On the basis of their elemental analysis **1**, **2**, **3**, **4**, and **5** complexes were formulated as Cu**L1**$Cl_2$, Cu**L1**$(NO_3)_2.3H_2O$, $Cu_2$**L2**$(NO_3)_4.4H_2O$, Zn**L1**$(CH_3COO)_2$, and $Zn_3$**L2**$(CH_3COO)_6.3H_2O$. The IR spectra of the complexes show secondary amine stretches in the 3225-3300 $cm^{-1}$ which are separated from OH stretching vibration. In the IR spectra of the nitrate complexes the absorption bands at 1400 and 1384 $cm^{-1}$ for **2** and **3**, respectively, are assignable to ionic nitrate groups.

## Crystal structure of 1

The molecular structure of the mononuclear complex Cu**L1**$Cl_2$ is given in Fig. 1 with selected distances and angles listed in the Table 2. Cu atom is five-coordinate having an $N_3Cl_2$ donor set. Cu1 is bound by three amine nitrogens from the macrocyclic ligand and two chlorido ligands. The degree of trigonality (τ) for Cu atom is 0.763 showing that geometry around copper is distorted trigonal bipyramidal with N2 and Cl1 as axial ligands [34]. The N2-Cu-Cl1 angle is 175.30(5) °. The Cu1-Cl1 bond distance in axial position (2.2610(5) Å) is shorter than of Cu1-Cl2 in equatorial position (2.6131(6) Å). The Cu-N bond distances (2.0330(15), 2.0370(16), and 2.0491(16) Å) are comparable to corresponding reported distances in references [35-37] although Cu1-N2 bond distance in axial position is the shortest.

The ligand forms two five-membered chelate rings involving the central Cu ion. The analysis indicated the Cu1-N1-C21-C20-N2 ring has a conformation twisted on C21-C20 and the Cu1-N2-C19-C18-N3 ring has a conformation twisted on C19-C18. Both chlorido ligands are positioned outside the macrocyclic ring. The presence of these chlorido ligands seems to enforce such a conformation that prevents any possibility of other donor atom being bound to the copper(II). The angle between the best planes of both phenolic rings is 62.58 deg.

Conformation of the macrocyclic ligand reported here results in N1-H1 group pointing towards the ring center. In such orientation, the group is a donor in three interactions to all oxygen atoms of the ligand as intramolecular hydrogen bonds, with the N1…O1, N1…O3 and N1…O2 distances being 2.866(2), 3.120(2) and 3.358(2) Å, respectively. Two other N-H groups are involved in the N-H…Cl interactions. In particular, N2-H2A

participates in a bifurcated bonds as intramolecular hydrogen bond (N2…Cl2 distance being 3.1829(17) Å) and intermolecular hydrogen bond (N2…Cl2 [-x, -y + 1, -z] distance being 3.3502(16) Å). The intermolecular weak interaction is formed by N3-H3A group, with the N3…Cl1 [-x+1,-y+1,-z] distance of 3.5649(17) Å and intramolecular hydrogen bond N3…Cl1 with 3.115 Å distance. The hydroxyl O2-H2 forms an intermolecular hydrogen bond to Cl2 of other molecule forming 1D hydrogen bonds and the O2…Cl2 distance is 3.2016(16) Å.

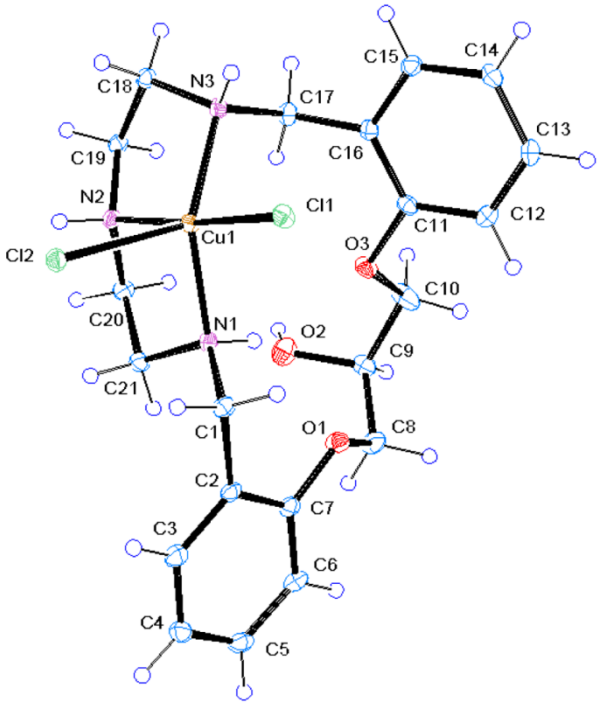



**Fig. 1.** The molecular structure of **1**

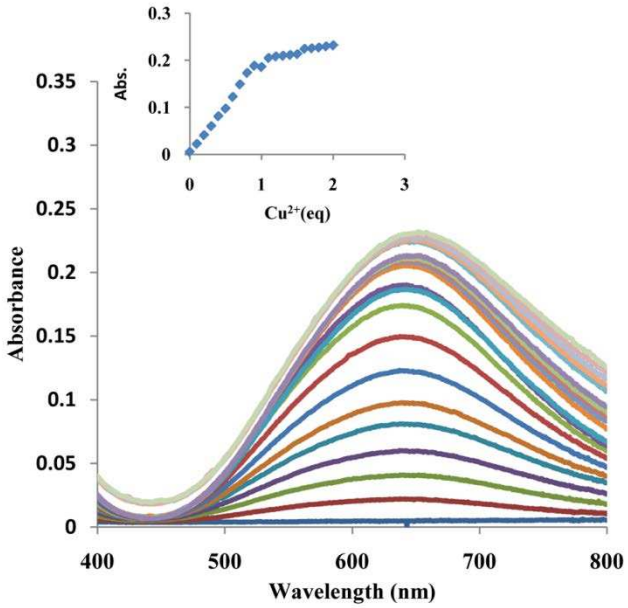
**Table 2**

Selected distances /Å and bond angles /° for complex **1**

| | |
|---|---|
| Cu1-N1 | 2.0491(16) |
| Cu1-N2 | 2.0330(15) |
| Cu1-N3 | 2.0370(16) |
| Cu1-C11 | 2.2610(5) |
| Cu1-C12 | 2.6131(6) |
| N2-Cu1-N3 | 82.92(6) |
| N1-Cu1-N2 | 84.61(6) |
| N1-Cu1-N3 | 138.90(7) |
| N2-Cu1-C11 | 175.30(5) |
| N3-Cu1-C11 | 92.75(5) |
| N1-Cu1-C11 | 99.89(5) |
| N2-Cu1-C12 | 85.52(5) |
| N3-Cu1-C12 | 123.99(5) |
| N1-Cu1-C12 | 93.70(5) |

**Complexation of macrocycles L1 and L2 with Cu(II) in solution.** Stochiometries of complexes formed by **L1** and **L2** with Cu(II) in methanol solution were determined under the same condition. The reaction equilibria involving macrocyclic ligands and copper(II) nitrate in methanol have been followed spectrophotometrically by observing the spectral changes that occur on the incremental addition of the metal ion to the ligand solution till no further change is seen. Ligands **L1** and **L2** show no absorption band in 400-800 nm region. Upon addition of a $Cu(NO_3)_2.3H_2O$ solution to a solution of ligands new bands appear in the spectra that are different from the spectra of the free ligands and free metal salt (Fig. 2 and Fig. 3). The variation of absorption at 646.4 nm as a function of equivalents of $Cu^{2+}$ is shown as insets in Fig. 2 and 3. For **L1**, the observed data indicate clearly a formation of a 1:1 metal to ligand species whereas the data for **L2** are

298 in accord with the initial formation of a 1:1 species followed by its conversion to a 2:1
299 species. These results are consistent with elemental analysis results in solid state.




300

301 **Fig. 2:** UV-Vis titration of **L1** ($1.25 \times 10^{-3}$ M) with various equivalents of $Cu(NO_3)_2.3H_2O$
302 in MeOH. The inset shows the variation of absorbance at 646.4 nm with number of
303 equivalents of $Cu^{2+}$

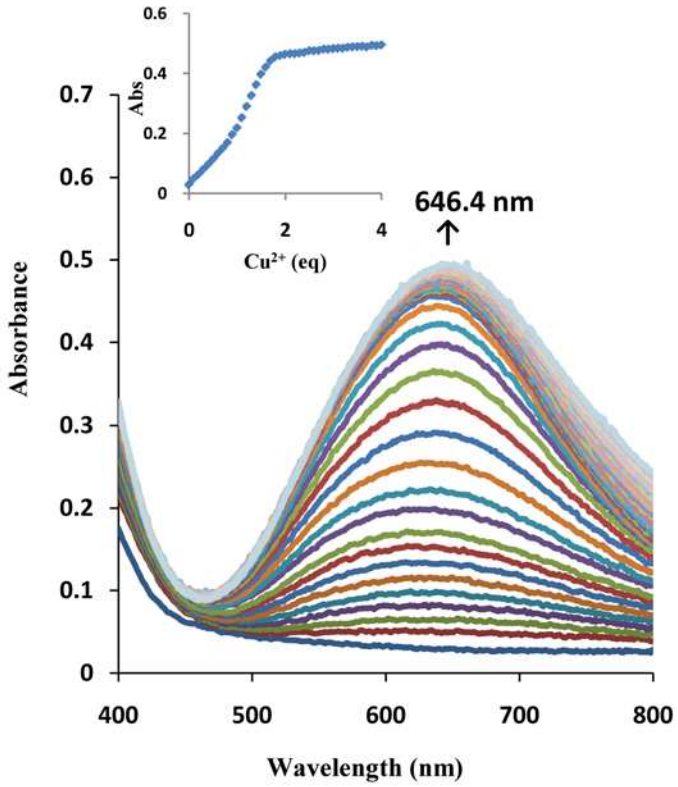



**Fig. 3:** UV-Vis titration of **L2** ($1.25 \times 10^{-3}$ M) with various equivalents $Cu(NO_3)_2.3H_2O$ in MeOH. The inset shows the variation of absorbance at 646.4 nm with number of equivalents of $Cu^{2+}$

**Cleavage of plasmid DNA.** The DNA cleavage activity of copper complexes **2** and **3** has been studied under physiological pH and temperature by gel electrophoresis by using supercoiled pG2 plasmid DNA as the substrate. The mononuclear copper complex **2** converted supercoiled DNA (Form I) to nicked form (Form II) and linear form (Form III) at ≤ 0.3 mM concentration of **2**, at 1 mM concentration only the linear and smear of small pieces of DNA were found (Fig 4a). For complex **3** the supercoiled DNA was completely degraded into linear form (Fig 4b, lane 2). At a higher concentration of **3** complex ($\geq 0.5$ mM), the linear DNA degraded completely into small pieces and smear

found (Fig 4b, lane 6, 7). These results indicated that dinuclear copper(II) complex **3** had dramatically higher activity than the mononuclear copper(II) complex **2** for the degradation of DNA plasmid.

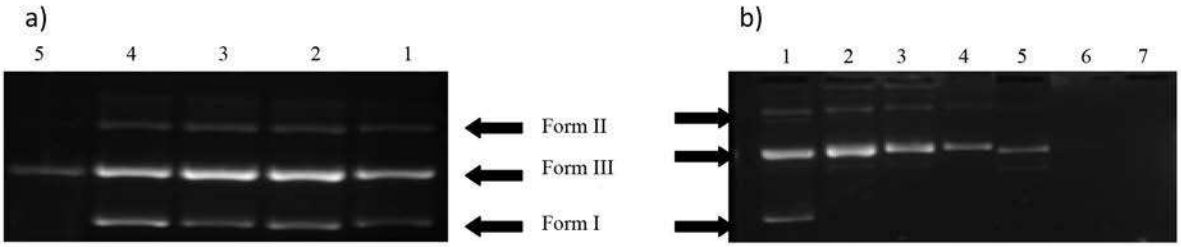
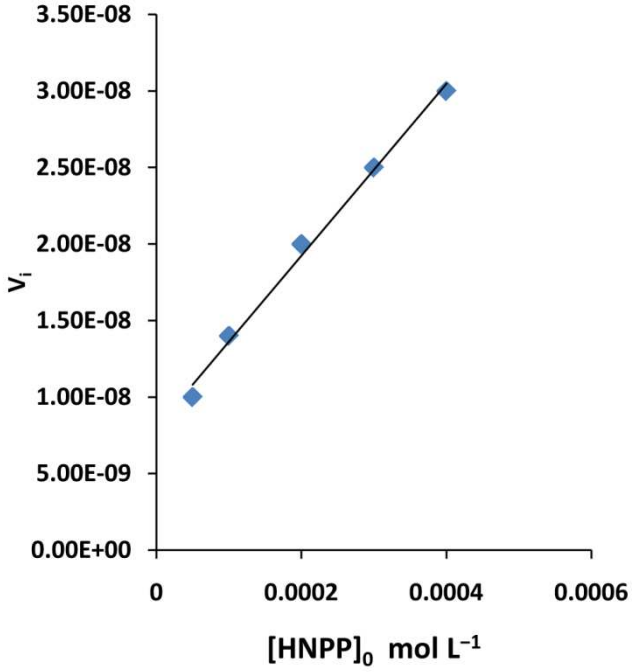



**Fig 4:** Agarose gel electrophoresis patterns for the cleavage of pGS2.plasmid DNA (650 ng/µL) by complexes **2** and **3** in the presence of 200-fold excess of MPA in the dark for 16 h in cacodylate buffer (0.1 mm, containig 50 mM NaCl, pH 7) 37 ˚C: **a)** Lane 1, DNA + MPA; lane 2, DNA + MPA + **2** (0.1 mM); lane 3, DNA + MPA + **2** (0.2 mM); lane 4, DNA + MPA + **2** (0.3 mM); lane 5, DNA + MPA + **2** (1 mM). **b)** lane 1, DNA + MPA; lane 2, DNA + MPA + **3** (0.1 mM); lane 3, DNA + MPA + **3** (0.15 mM); lane 4, DNA + MPA + **3** (0.25 mM); lane 5, DNA + MPA + **3** (0.35 mM); lane 6, DNA + MPA + **3** (0.5 mM); lane 7, DNA + MPA + **3** (1 mM).

**Phosphodiester hydrolysis.** Zinc(II) complexes of **L1** and **L2** were soluble in aqueous buffered solutions, therefore we could study the hydrolysis of HNPP by them. The efficiency of trinuclear zinc(II) complex **5** was studied at three different pH values (5, 7, and 8.5) with respect to HNPP hydrolysis and compared to the activity of mononuclear complex **4**. While an important effect of pH on the HNPP hydrolysis was observed for **5**, the mononuclear complex **4** exhibited no activity at the chosen three pH values. For **5** we observed activity only at pH 8.5. Therefore, the hydrolysis of HNPP by **5** was

followed by the visible absorbance change. Initial velocities ($V_i$) were measured by following the absorbance at 400 nm due to the release of 4-nitrophenolate anion. The blank hydrolysis process without the complex as promoter was studied at pH = 8.5 for 0.05 – 0.4 mM HNPP concentrations. The observed rate constant for uncatalytic ($k_{uncat}$ = 6 × $10^{-5}$ 1/s) was calculated from the slope of the straight line ($V_i$) versus $[HNPP]_0$ (Fig. 5). The results indicate that the rate is first order with respect to substrate. Rate constant value of catalytic hydrolysis ($k_{cat}$ = 2.1 × $10^{-3}$ 1/s) was obtained for constant concentration of **5** (0.15 mM) for (0.05 – 0.9 mM) HNPP concentrations (Fig. 6). Therefore, under identical conditions, a $k_{cat}$ / $k_{uncat}$ ≅ 35 fold increase in hydrolysis rate over HNPP self – hydrolysis was observed, when trinuclear zinc complex catalyst added to HNPP solution. To understand whether the cooperation consists between zinc(II) ions for trinuclear complex in catalytic hydrolysis, we examined the rate constant of mononuclear Zn(II) complex. Almost no hydrolysis rate enhancement was observed when mononuclear zinc complex was added.




350

351 **Fig 5.** $V_i$ vs. $[HNPP]_0$ hydrolysis of HNPP without catalyst. Each reaction mixture
352 contained 10mM Cacodylate buffer/ 0.1 mM KCl, pH 8.5 at 40˚C and (0.05, 0.1, 0.2, 0.3,
353 0.4 mM) HNPP. The plotted lines are the computer-generated best fit with R = 0.9938

354

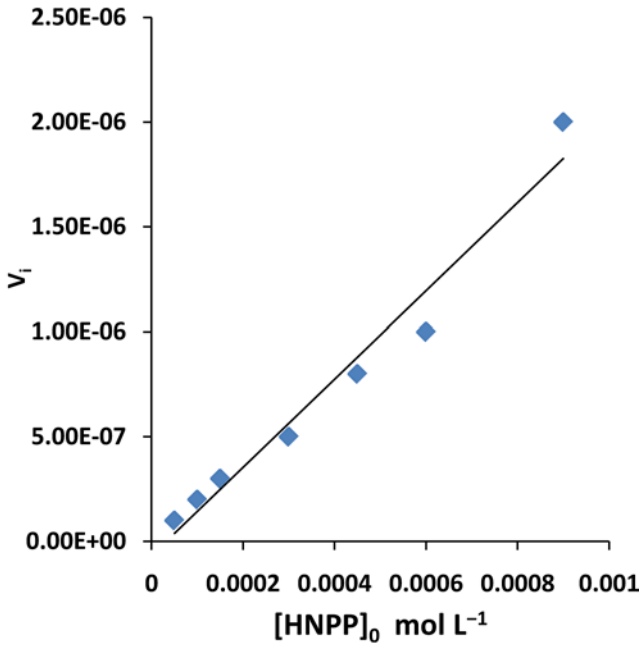



**Fig 6:** $V_i$ vs. $[HNPP]_0$ of hydrolysis of HNPP (0.05-0.9 mM) by **5**. Each reaction mixture contained 10 mM Cacodylate buffer / 0.1 mM KCl, pH 8.5 at 40 ˚C and 0.15 mM **5**. The plotted lines are the computer-generated best fit with R = 0.966

## Conclusions

In conclusion, we have presented the successful and clean synthesis of the new tri-linked macrocycle **L2** using simple protecting group strategy and Williamson etherification chemistry. This work is a good example of the design of multinuclear complexes for artificial nucleases and DNA cleavage. The trinuclear zinc(II) complex **5** displayed good hydrolytic activity for phosphate diester, which could be attributed to polynuclear structure of the **5** at pH 8.5. Mono and dinuclear copper(II) complexes cleaved plasmid pG2 DNA and dinuclear copper(II) complex **3** showed much higher cleavage efficiency than their mononuclear analogue **2** at the same $Cu^{2+}$ concentration.

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## Appendix A. Supplementary material

CCDC 967934 contains the supplementary crystallographic data for Cu**L1**$Cl_2$ complex. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated $^{1}H$, $^{13}C$ NMR, DEPT $^{13}C$ NMR and MS of **L2**.

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